REMARKS

I. Status of the claims and application

Claims 1-10 and 12-18 are pending. Claims 1, 2, 8, 9, 13, 14, and 16 have been amended.

Applicants added a sentence to the beginning of the application stating that the present application is filed under 35 U.S.C. § 371 from PCT/FR98/02042, filed September 23, 1998.

Furthermore, and pursuant to the examiner's instruction, applicants also have provided an abstract on a separate sheet appended to this paper.

The examiner states that "applicant has not filed a certified copy of the French application." However, the Notice of Acceptance mailed June 14, 2000, indicates in section 4. that the priority document has been received. Accordingly, applicants have not provided another copy of this document.

Applicant has submitted with this amendment copies of all U.S. patents that were not filed with their previous Information Disclosure Statement.

II. Summary of the Invention

The present invention envisions a "microspore-specific" promoter and a method for producing hybrid plants with "gametophytic male sterility." The invention uses "a promoter region which controls the expression, specifically in the microspores, of a gene encoding a cytotoxic molecule." Specifically, the invention employs the promoter region of the nucleotide sequence denoted as SEQ ID NO. 3 to drive expression of another nucleic acid "specifically in the microspore." See page 3, lines 14-18. The promoter region of SEQ ID NO. 3 "is defined as being the sequence preceding (on the 5' side [of]) the translation start codon." See page 3, lines 28-31.

A vector construct of the present invention, therefore, comprises **the promoter region of SEQ ID NO. 3** fused to "another [open reading frame] whose product is a

cytotoxic molecule [which is] capable of destroying" microspores. See page 4, lines 4-10, and page 6, lines 27-31. The toxicity of such a molecule can also be controlled according to the present invention. See page 2 of the specification at lines 33-37 and, similarly, page 3, lines 1-2. For instance, the cytotoxic effects of a "substilisin," when fused to the promoter region of SEQ ID NO. 3, can be inhibited by applying flurophosphate insecticide to the plant. See page 7, lines 7-13.

III. The Office Action

The examiner rejected claims 1-10 and 12-18 under Section 112, paragraph 1; claims 3-4, 7, 9, and 13 under Section 112, paragraph 2; claims 1-3, 5-9, and 13-14 under Section 102(e); claims 1-3, 5-9, and 12-17 under Section 102(e); claim 18 under Section 102(a); claim 14 under Section 101; claims 1 and 2 under Section 101; and claim 4 under Section 103(a).

IV. Overcoming the Examiner's Rejections

(i) The present claims are enabled

The examiner rejected claims 1-10 and 12-18, under Section 112, first paragraph, alleging that applicants do not identify, demonstrate, specify, nor describe (a) "the structural features unique to SEQ ID NO. 3"; (b) "the functional domains of the sequence"; (c) "the overall function of the sequence"; (d) "a gene encoding a male-gamete-specific cytotoxic product"; (e) "a substilisin protease"; (f) "that SEQ ID NO. 3 or sequences that are 80% homologous or that hybridize under conditions of unspecified stringency to SEQ ID NO. 3 or nucleotides 1 to 2111 of SEQ ID NO. 3 or sequences that are 80% homologous or that hybridize to said subsequence of SEQ ID NO. 3 will still specify expression in microspores"; (g) "that operably linking one of these sequences to a gene encoding a protease or subtilisin will disrupt the normal development of the microspores"; or that (h) "a promoter operably linked to a cytotoxic product can be induced by applying to the plant an insecticide molecule of the fluorophosphates family." See pages 3-8 of the Office Action.

By using a microspore-specific clone isolated from *B. napus*, to screen a commercially available microspore cDNA library, applicants identified "clone BnM3.4," *i.e.*, "SEQ ID NO. 3" the nucleic acid sequence encoding a microspore-specific gene from *Brassica*

napus, *i.e.*, "rape." Applicants provide ample description in the present application for (a) "the structural features unique to SEQ ID NO. 3"; (b) "the functional domains of the sequence"; and (c) "the overall function of the sequence." See page 3, lines 14-37; page 4, lines 1-10; page 11, lines 23-25; Figure 2; the legend for Figure 2 at page 9, lines 22-31.

It is clear from reading those portions of the specification that SEQ ID NO. 3 includes a promoter regulatory element between nucleotides 1 and 2111, and that this region "is defined as being the sequence preceding (on the 5' side [of]) the translation start codon (ATG)" page 3, lines 28-31. "The largest envisagable promoter region concerning SEQ ID NO. 3 stretches from nucleotide 1 to nucleotide 2111, and preferably from nucleotide 1 to 2084." See page 4, lines 1-3. The nucleotide structure of SEQ ID NO. 3, including the "most valuable part," *i.e.*, the promoter region, is presented in Figure 2. As stressed above, the present invention contemplates the use of the promoter of a gene expressed specifically in the microspore, such as the promoter region spanning 1-2111 of the gene depicted in SEQ ID NO. 3, to drive the expression of a cytotoxic gene whose product prevents the creation of viable pollen.

The structural features unique to SEQ ID NO. 3 include the entire sequence of clone BnM3.4, as described in Figure 2. Applicants assert that the protein sequence of clone BnM3.4 is not identical to any protein described "in the databanks." There is no requirement that applicants must provide the exact "domain" parameters that render the promoter functional; only that applicants describe a nucleic acid sequence known to be a "promoter." The region of a gene upstream from the ATG start codon is regarded by the skilled artisan as the promoter-bearing portion of the gene. Applicants explicitly state throughout the application that the upstream, *i.e.*, 5', region of SEQ ID NO. 3 exemplifies a microspore-specific promoter sequence.

Applicants go so far as to show in Example 2, "B/Transformation with a chimeric gene" at page 12 of the specification, that nucleotides 1 to 2056 of SEQ ID NO. 3 were used to drive expression of the β-glucoronidase gene. The results showed that the microspores of plants transformed with such a construct were blue in color, indicating that β-glucoronidase was expressed specifically in the microspore. "No coloration is present in the adjacent tissues of the anther, nor in the somatic tissues of the plant." See page 13, lines 7-12. Applicants conclude that "the specificity of expression conferred by this 2 kb promoter" is directed to the cells constituting the microspores of the transformed plants' anthers.

Accordingly, applicants teach features that constitute the "structural features that are unique to SEQ ID NO. 3"; define a functional domain of SEQ ID NO. 3 as being the promoter region, *i.e.*, approximately 2 kb upstream of the start codon; and that the "overall function" of the promoter of SEQ ID NO. 3 is to drive expression of a gene specifically in the cells of the microspore of a transformed plant.

The examiner also believes that the specification must provide a description of (d) "a gene encoding a male-gamete-specific cytotoxic product and also (e) "a substilisin protease." Applicants are under no such obligation. The present invention does not relate to a "male-gamete-specific cytotoxic product." The present invention relates to the use of a microspore-specific promoter, or other similar regulatory element, to express a gene capable of inhibiting the creation of pollen. According to the present invention, the product of that gene destroys the capability of that microspore to create pollen. Any cytotoxic gene may be linked to a microspore-specific promoter. See page 4, lines 4-10. In the case of the present invention, the cytotoxic gene is one that encodes a protease, preferably subtilisin, such as that of *Bacillus amyloliquefasciens*. See page 3, lines 11-24 of the specification.

The skilled artisan knows that genes encoding proteases such as subtilisin can be operably linked to the promoter of SEQ ID NO. 3 and subsequently expressed in microspores. Indeed, the skilled artisan is aware of a myriad other proteases that may be employed according to the rationale of the present invention. It is not necessary for applicants to list in the specification each and every protease that may be operably linked to their microspore-specific promoter. It is enough that applicants have stated that "subtilisin" is one particular type of protease that one may use to practice the invention. Furthermore, applicants teach that the subtilisin isolated from *Bacillus amyloliquefasciens* is particularly useful. Moreover, THE AMERICAN HERITAGE DICTIONARY defines "subtilisin" as "an extracellular enzyme produced by certain strains of a soil bacterium (*Bacillus amyloliquefaciens*) that catalyzes the breakdown of proteins into polypeptides and resembles trypsin in its action." See Exhibit 1.

One of skill in the art would know, therefore, from reading the present specification and from their general understanding of the art, that any one of a variety of cytotoxic-encoding genes may be used according to the present invention, including proteases, and more specifically, substilases, to disrupt viable microspore production.

With that reasoning, one of skill in the art would know that the function of a protease is to degrade, cut, and generally catalyze the breakdown of proteinaceous molecules. In the context of the present invention, however, this protease is "cytotoxic" because it "produces a toxic effect on cells" (THE AMERICAN HERITAGE DICTIONARY, see Exhibit 2). That "toxic effect" is the resultant inability of transformed microspore cells to produce pollen when a protease is expressed in its cells. Accordingly, the examiner's contention that applicants have not taught (g) "that operably linking one of these sequences to a gene encoding a protease or subtilisin will disrupt the normal development of the microspores" is unfounded. Subtilisin and it's effects upon proteins are well known. One would expect that a subtilisin produced in a cell would act to breakdown proteins, especially those containing serines.

The examiner also contends that applicants do not show that "SEQ ID NO. 3 or sequences that are 80% homologous or that hybridize under conditions of unspecified stringency to SEQ ID NO. 3 or nucleotides 1 to 2111 of SEQ ID NO. 3 or sequences that are 80% homologous or that hybridize to said subsequence of SEQ ID NO. 3 will still specify expression in microspores." Applicants do not have to provide experimental results for all permutations of their invention. It is sufficient that applicants teach that one may use the promoter region of a gene that is specifically expressed in the microspore to drive expression of a cytotoxic product that prevents the microspore from producing viable pollen. To that end, and as has been elaborated upon above, applicants teach how to construct a transformation vector comprising the promoter region of SEQ ID NO. 3 and relates how that construct expresses a gene to which it is operably linked (the "GUS" gene) specifically in the microspores.

The skilled artisan has the wherewithal to realize that applicants' invention requires the use of a nucleotide sequence that is capable of expressing a second nucleotide sequence to which it is operably linked. Accordingly, it is well within the purview of the skilled artisan to obtain, select, or design a nucleotide sequence from SEQ ID NO. 3 that is 80% homologous in sequence or is a fragment of SEQ ID NO. 3, that is capable of expressing a second nucleotide sequence. Since applicants demonstrated that nucleotides 1-2056 were capable of just such expression, they have demonstrated that the present invention is enabled.

The examiner also states that "applicants have not taught or demonstrated that a promoter operably linked to a cytotoxic product can be induced by applying to the plant an

insecticide molecule of the fluorophosphates family." Applicants respectfully point out that the examiner has misinterpreted the present invention. The insecticide molecule does not *induce* promoter activity, but rather, accomplishes the opposite effect. The step of applying an insecticide to a transformed plant is to inhibit the cytotoxic product. "When the cytotoxic product is a subtilisin . . . the inhibition is achieved by the action on the transformed plant of an insecticide molecule . . . indeed, this molecule is capable of restoring the total fertility of the hemizygous plants by inhibition of subtilisin." See page 7, lines 7-21 of the specification.

Accordingly the present claims are free from objection and applicants respectfully request that the examiner withdraw the rejections.

(ii) The claims are not indefinite

The examiner rejected claims 3-4, 7, 9, and 13 because he believes that applicant has not defined "cytotoxic product." Similarly, the examiner contends that applicant has not defined or described a cytotoxic product that is specific to plant male gametes. The examiner also states that the wording of claim 9 is unclear and that claim 13 refers to methodological steps in claim 9, from which it depends, but that claim 9 had been amended to delete these steps.

Applicants have defined "cytotoxic product." See, for instance, page 4, lines 4-10, where applicants define a cytotoxic product as "capable of destroying only said microspores." Such a product may be a protease, such as a subtilisin. See page 4, lines 15-24. Applicants do not seek to use a cytotoxic product that is specific to plant male gametes. Rather, the promoter region of SEQ ID NO. 3 directs the expression of any gene to which it is operably linked to the plant male gametes, *i.e.*, to the microspore. The present invention does not require the use of a plant male gamete-specific cytotoxic product, only that the cytotoxic product is fused to a microspore-specific regulatory element, such as the promoter region of SEQ ID NO. 3.

Applicants have amended claims 9 and 13 to overcome the examiner's rejections.

Accordingly, applicants request that the examiner kindly withdraw the rejections.

(iii) The claims are not anticipated under Section 102(e) by Cigan *et al.*, Sim *et al.*, and Mariani *et al.*

The examiner holds that Cigan *et al.* (United States Patent No. 5,689,049) teaches "transforming a plant with an expression vector comprising a methylase gene operably linked to a promoter that specifically directs expression in the anthers."

The examiner also contends that Mariani *et al.* (United States Patent No. 5,689,041) and Sim *et al.* (United States Patent No. 5,993,827) anticipate the present invention because "Mariani *et al.* teach a barnase gene which encodes a cytotoxic product, which comprises a fragment thereof that reads on applicant's claimed sequences, as a fragment can be interpreted as comprising one base pair of DNA." See page 12 of the Office Action. The examiner also states that Sim *et al.* teach a DNA sequence "that exhibits 3.6% identity with SEQ ID NO. 3"; and that, "given the wording of the claim, i.e., a sequence that hybridizes under unspecified conditions to SEQ ID NO. 3 or a fragment thereof, any sequence that shares at least one base pair with SEQ ID NO. 3 or derivatives thereof, would be encompassed by the claim." See page 11 of the Office Action.

Applicants respectfully disagree. Cigan teaches a sporophytic promoter that is active before or during male meiosis. According to Cigan, hemizygote transgene plants containing DAM-methylase expressed from the methylase gene driven by the sporophytic promoter are totally male-sterile. That is not the outcome obtained when one uses the promoter of the present invention. In fact, the present invention uses a gametophytic promoter, *i.e.*, it is active in male gametophytes and, therefore, allows expression of a co-joined polynucleotide or gene, *after* meiosis, unlike the promoter described by Cigan.

Furthermore, and contrary to the examiner's rationale, "one base pair of DNA" is **not** a "promoter" or a nucleic acid fragment that is capable of expressing a gene to which it is fused, in a microspore. A "fragment" of the present invention, *i.e.*, of SEQ ID NO. 3, is one that is capable of expressing a gene to which it is operably linked, in the microspore cells of a transformed plant. From reading the specification, one would understand that that "fragment" is the promoter or a part of the promoter of SEQ ID NO. 3. The inventive methodology requires transformation of plant with a vector "comprising a microspore-specific promoter sequence placed upstream of a gene encoding a cytotoxic product." See

page 6, lines 27-31. The amended claims recite such functionality, and applicants respectfully request that the examiner withdraw these rejections.

(iv) The claims are not anticipated under Section 102(a) by Ballinger *et al.*, 1996

The examiner asserts that Ballinger *et al.* teach a vector comprising a nucleotide sequence encoding a subtilisin operably linked to a promoter for expression in *E. coli* wherein the promoter would comprise a fragment of the claimed invention given that a fragment can be defined as a single base pair, and as such, Ballinger *et al.* anticipate the claimed invention.

Applicants respectfully disagree with the examiner's allegation for the same reasons applicants use to overcome Mariani *et al.* A "single base pair" does not constitute a "fragment" of SEQ ID NO. 3 that is capable of expressing a gene to which the fragment is operably linked.

Accordingly, Ballinger *et al.* does not teach each and every limitation of the present claims. For at least this reason, applicants request that the rejection be withdrawn.

(v) The present claims are not obvious under Section 103(a)

The examiner alleges that claim 4 is unpatentable over Mariani *et al.* in view of Ramjee *et al.*, 1996. The examiner states that Ramjee teaches an isolated nucleic acid encoding a papain protease from papaya fruit than can be used in the method of Mariani *et al.* The examiner admits that Mariani *et al.* does not teach a gene encoding a protease. The examiner relies on Mariani *et al.* to teach a fragment of the promoter of the present invention, because, according to the examiner, a single nucleotide base pair is a "fragment." Thus, "it would have been obvious to use the method of Mariani *et al.* and to modify this method by using a protease, such as papain, as taught by Ramjee *et al.*"

For the reasons cited above, the present claims are not obvious in light of the prior art. The skilled artisan would not be motivated to take a single nucleotide base pair from Mariani *et al.* and fuse to it the protease of Ramjee *et al.*, on the assumption that that construct could be successfully used to produce non-viable pollen in the microspore of a plant.

The claims of the invention are not anticipated or rendered obvious by the combination of Mariani *et al.* and Ramjee *et al.* Accordingly, applicants respectfully request that the examiner withdraw this rejection.

(vi) The present claims are directed to statutory subject matter

The examiner contends that, in claim 14, it is "unclear whether the claimed seeds would be distinguishable from seeds that would occur in nature" and suggests that amending the claims to recite that "the seeds comprise the construct that was introduced into the parent seed" would overcome the rejection.

Accordingly, applicants have amended claim 14 to reflect that the seed comprises the inventive construct.

The examiner also contends that claims 1 and 2 are directed to non-statutory subject matter because they read on "naturally occurring nucleic acid since it has not been isolated. The examiner suggests that amending claims 1 and 2 to recite "An isolated nucleic acid sequence" would overcome this rejection.

Applicants have amended claims 1 and 2 to recite that the nucleotide sequence is a *purified* nucleotide sequence. Accordingly, the rejections no longer apply and applicants respectfully request that the rejections be withdrawn.

IV. Conclusion

In view of the foregoing, applicants submit that the present claims are free from objection and earnestly solicit an early notice of allowance. Nevertheles, should there be any questions, Examiner Baum is courteously invited to contact the undersigned attorney at the telephone number shown below.

Respectfully submitted,

Date: Dec. 13, 2002

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MARKED-UP VERSION OF THE CLAIMS

1. (Twice amended) A purified n[N]ucleotide sequence comprising:

a) at least a part of SEQ ID NO. 3, or

[b) a sequence which hybridizes to the sequence according to a), or]

b[c]) a sequence which has at least 80% homology with a) [or b) or

d) a sequence which is a fragment of a)],

wherein said purified nucleotide sequence is capable of expressing a second nucleotide sequence to which it is operably linked.

2. (Twice amended) A purified n[N]ucleotide sequence according to claim 1, comprising:

a) a nucleotide sequence comprising [which stretches from] nucleotides 1 to [nucleotide] 2111 of SEQ ID NO. 3, or

[b) a nucleotide sequence which hybridizes to the sequence according to a), or]

b[c]) a nucleotide sequence which has at least 80% homology with a) [or b)] or

c[d]) a nucleotide sequence which is a fragment of a) or b),

wherein said purified nucleotide sequence is capable of expressing a second nucleotide sequence to which it is operably linked.

8. (Amended) A m[M]ethod for producing a plant with gametophytic male sterility with inducible fertility, comprising[:] inserting into one or more plant cells a gene present in a construct, wherein the expression product of said gene is cytotoxic to a microspore; and producing a plant therefrom which does not produce a male gamete.

9. (Amended) A m[M]ethod according to Claim 8, wherein said gene is inserted into [as] a [a] vector which comprises [comprising] a nucleotide sequence, wherein said nucleotide sequence comprises (i) the sequence which stretches from nucleotide 1 to nucleotide 2111 of SEQ ID No. 3, or (ii) a sequence which hybridizes to the sequence according to (i), or (iii) a sequence which has at least 80% homology with (i) or (ii), or a sequence which is a fragment of (i), wherein said sequence is upstream of a DNA sequence encoding a cytotoxic product; and further comprising inhibiting the cytotoxicity of the gene product, thereby inducing the fertility of the plant; self-fertilizing the fertile plant; and selecting any plants which do not produce male gametes.

12. (Amended) A plant according to claim 7, wherein said plant belongs to the *Brassicaceae* family.

13. [A] The method according to claim 9, further comprising[:] multiplying the plants which do not produce male gametes [obtained in step d) by reproducing steps b) and c)].

14. (Amended) A seed derived from the plant obtained by the method according to claim 8, wherein said seed comprises said construct.

16. (Twice amended) A plant obtained by the method of claim 8, wherein said plant belongs to the *Brassicaceae* family.